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Electrophoresis Studies on Blood Plasma Esterases

II. Avian, Reptilian, Amphibian and Piscine Plasmata

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The esterase activity of the plasmata from birds, reptiles, amphibians and fish was investigated using as substrates a series of aliphatic, aromatic, heterocyclic and choline esters. The distribution of esterase activity and total protein after electrophoresis on cellulose columns are reported for the plasmata of cock, duck, turtle, two frogs (*Rana, Xenopus*), and five teleostian fishes (pike, cod, eel, wrasse, frogfish). The electrophoretic patterns for the plasma proteins of one Elasmobranch (*Squalus*) and one Cyclostome (*Myxine*) are also demonstrated.

Of the three types of esterases present in most mammalian plasmata, only the aliesterases (B-esterases) and cholinesterases (C-esterases) were present in the plasmata of lower vertebrates, with the single exception of the plasma from female frogs which also contained an arylesterase (A-esterase) in addition to a propionylcholinesterase. Cock and chicken plasmata were characterized by a propionylcholinesterase which was the only esterase present and differed from other cholinesterases in many respects. Duck plasma contained a propionyl-B-esterase in addition to a propionylcholinesterase. One esterase only was present in turtle plasma; it had the characteristics of both a B- and C-esterase and was highly sensitive to physostigmine although non-choline esters were hydrolysed more rapidly than choline esters. More than one B-type esterase were probably present in the plasma of *Xenopus laevis*, which also contained a butyrylcholinesterase in low concentration.

In all the teleostian plasmata, an acetylcholinesterase was present in low concentration. The main esterases present in these plasmata were acetyl- or propionyl-B-esterases. Eel plasma contained such an esterase in very high concentration. The plasma of spiny dogfish had low B- and C-esterase activities, and the esterase activity of hagfish plasma with most substrates was negligible.

The esterase patterns of mammalian plasmata were discussed in the first paper of this series [1]. This paper considers the plasmata from lower vertebrates from a comparative point of view.

MATERIAL AND METHODS

Plasmata were prepared from heparinized blood, obtained by heart puncture or by cutting the ventral aorta (fish).

Substrates and inhibitors were the same as in the first report of this series. The following abbreviations are employed: acetyl, Ac; propionyl, Pr; butyryl, Bu; benzoyl, Bz; succinyl, Su; *iso*amyl, Am; triglycerides, *e. g.*, TBu; phenyl, Ph; choline, Ch; acetyl-β-methylcholine, MeCh; polyoxyethylene sorbitan monolaurate, Tween 20; tetra-*iso*propylphosphoramide, *iso*-OMPA; bis-mono*iso*propylamine fluorophosphine oxide, mipafox; 10-(1-diethylaminopropionyl) phenothiazine hydrochloride, Astra 1397; 1,5-*bis* (4-trimethylammoniumphenyl) pentane-3-one diiodide, 62C47; *bis*- (piperidinomethyl coumaranyl-5)ketone, 3318CT.

Electrophoresis was carried out on cellulose columns (3 cm $\times$ 40 cm) in veronal buffer solution (pH 8.4, $I = 0.1$) at 5–15°C, using 5 ml of buffered plasma [1]. The protein concentration of each eluted fraction (2.8 ml) was estimated by a modified Folin procedure [2].

Esterase activity was determined by the Warburg technique and expressed in b_{30} values [3]. The efficiency of esterase inhibition by various compounds was expressed in pI_{50} values, extrapolated from curves of inhibition (%) — inhibitor concentration (pI) where sufficient inhibitor was used at the higher pI levels to establish the point of complete inhibition.

RESULTS

The hydrolysis rates of various substrates by original plasma samples are summarized in Table 1. Activity values (b_{30} per 0.1 ml plasma) obtained with additional substrates are given in the text. The electrophoretic patterns presented in Figs. 1—11 show the rate of elution of the esterases in relation to various protein constituents. In the legends to these figures, relative substrate specificity and sensitivity to certain inhibitors of each esterase component are summarized in tabular form. The peaks of esterase activity are designated B and C, corresponding to aliesterase and cholinesterase activities, respectively. Arylesterases (A) were not present in the plasmata studied, except for a sample from frog (*Rana*).

Cock and chicken (Fig. 1). The esterase pattern of cock plasma was characterized by the comparatively high hydrolysis rate of propionylcholine which was the highest value observed among the esters used as substrates. This plasma was unique among those examined in being devoid of both A- and B-esterases. The B-type esterase described by Aldridge [4] was actually identical with the propionylcholinesterase present, hydrolysing acetyl-β-methylcholine at a comparatively high rate. This enzyme was a typical physostigmine-sensitive propionylesterase hydrolysing both tripropionin and phenyl propionate at a higher rate than their other acyl homologues. No other esterases were detected on electrophoresis in the separated plasma. The hydrolysis rates of four different substrates were all found to be influenced at the same degree by the eight inhibitors tested (Table 2), providing additional evidence that the same single esterase hydrolysed these substrates. This esterase was found to be extremely sensitive to mipafox.

The propionylcholinesterase of the cock differed from both the acetylcholinesterases present in most animal erythrocytes and from the butyrylcholinesterases present in several mammalian plasmata. This difference included the unusual substrate specificity, *i.e.*, high rate of hydrolysis of acetyl-β-methylcholine (also observed previously [5,6]) and various types of non-choline esters,

Table 1. Enzymic hydrolysis of various esters by avian, reptilian, amphibian and piscine plasmata. Samples from at least three animals of each species were analysed. The activity values (b_{30}) refer to initial hydrolysis rates with a representative sample and are expressed in μl CO_2 per 0.10 ml of plasma per 30 min, corrections made for nonenzymic hydrolysis. Where values are indicated in brackets, initial hydrolysis rate was determined by extrapolation. A question mark indicates that such extrapolation could not be done because of a too rapid decrease of the hydrolysis rate. An asterisk denotes that the activity varies greatly from individual to individual. Code names are explained in the text.

Species	AcCh	PrCh	BuCh	MeCh	BzCh	SuCh	AmAc	AmPr	AmBu	TAc	TPr	TBu	PhAc	PhPr	PhBu
Cock	37	71	36	19	2	<1	4	6	7	10	36	22	36	62	38
Duck	43	74	67	7	8	2	?	?	?	30	220	112	350	505	360
Turtle, *Testudo*	14	103	27	7	1	0	(450)	(500)	(300)	700	1 500	800	650	1 030	320
Frog, *Rana*	40	90	87	2	10	7	10	21	59	12	53	33	180*	265*	220*
Clawed frog, *Xenopus*	2	5	9	—	—	—	34	51	70	108	402	309	900	—	1 020
Pike, *Esox*	12	2	1	1	1	<1	(40)	(65)	(120)	120	400	280	370	—	255
Cod, *Gadus*	6	4	3	2	1	1	?	(4)	(4)	390	107	20	570	—	27
Eel, *Anquilla*	10	4	3	2	<1	21	?	?	?	800	4 250	4 750	8 000	—	20 300
Wrasse, *Labrus*	4	2	2	1	1	<1	5	(20)	16	28	160	130	160	—	345
Frogfisch, *Lophius*	13	6	4	2	5	3	10	7	6	523	175	31	770	—	33
Dogfish, *Squalus*	4	3	1	<1	<1	<1	2	2	2	3	10	4	14	—	11
Hagfish, *Myxine*	0	0	0	0	<1	0	0	0	0	0	0	0	<1	—	<1

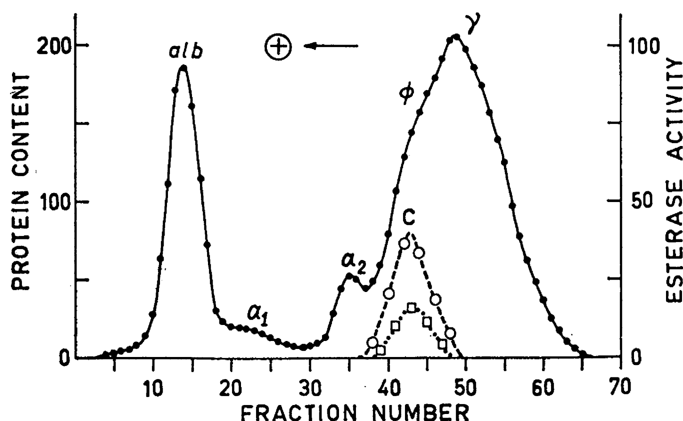

Fig. 1. Cock plasma. Distribution of esterase activity and total protein after column electrophoresis (veronal buffer, pH 8.4; $I = 0.1$; 260 V; 60 mA; 30 h). The protein content is expressed as relative amount based on extinction values. Esterase activity is expressed in b_{30} values with the ml aliquots from each fraction as indicated below: ●——●, protein concentration; □·········□, PhAc (0.40) and TPr (0.40); O— — —O, PrCh (0.40).

Esterase activities (b_{30}/0.10 ml) against various substrates and esterase inhibition (pI_{50}) by physostigmine (Phys) and an organophosphorus compound of the peak fraction from each esterase component.

Esterase component	PhAc	PhPr	PhBu	TPr	AcCh	PrCh	pI_{50}	
							Phys	Mipafox
C	4	8	5	4	5	10	7.8	9.0

and a unique pattern of susceptibility to various esterase inhibitors since those being selective inhibitors of acetylcholinesterase (62C47 and 3318CT) had very little effect in this case (Table 2). The low inhibiting effect of such compounds on the chicken serum cholinesterase was observed previously [7,8]. Rat plasma contains a propionylcholinesterase based on substrate specificity, but the propionylcholinesterase of cock plasma differs from that of the rat in showing no inhibition by excess substrate (AcCh or PrCh), as was demonstrated also in previous papers [5,9]. The cock (chicken) plasma cholinesterase, therefore, has to be regarded as a special type of physostigmine-sensitive esterase, differing from the cholinesterases of other vertebrate plasmata in many respects. It is another example of the view [1] that no clear-cut distinction between various groups of cholinesterases can be made.

Chicken plasma gave the same results both regarding the properties of the propionylcholinesterase present and the lack of other types of esterases. The sex difference in plasma cholinesterase activity observed by Caridroit *et al.*[10] was not further investigated.

Table 2. Susceptibility of cock-plasma esterase to various inhibitors. Values refer to negative logarithm of molar inhibitor concentration producing 50 % esterase inhibition. The enzyme preparation (whole plasma and separated esterase fractions, respectively) was incubated for 50 min with the inhibitor prior to addition of substrate.

Inhibitor	Propionyl-choline	Tri-propionin	Phenyl-acetate	Phenyl-butyrate
Physostigmine	7.8	7.8	7.85	7.6
iso-OMPA	6.2	6.0	6.25	6.25
Mipafox	9.0	8.9	8.9	8.9
Astra 1397	5.3	5.4	5.25	4.55
3318CT	<3.5	<3.5	<3.5	<3.5
62C47	<3.5	<3.5	<3.5	<3.5
Atoxyl	<3.5	<3	<3	<3
Quinine sulphate	<3.5	<3.5	<3	<3

Duck (Fig. 2). The plasma from duck hydrolysed the phenyl esters and the triglycerides at a much higher rate than did cock plasma. With these substrates and with choline esters, the propionates were hydrolysed more rapidly than the corresponding acetates and butyrates. The hydrolysis rate with

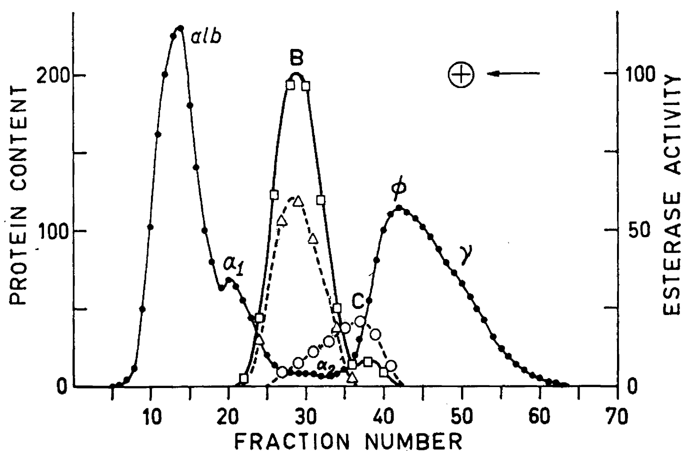

Fig. 2. Duck plasma. Conditions as for Fig. 1. □——□, PhBu (0.20) and PhPr (0.10); △– – –△, TPr (0.20); ○– – –○, PrCh (0.40).

Esterase component	PhAc	PhPr	PhBu	TPr	PrCh	BuCh	pI_{50}			
							Phys	*iso*-OMPA	Astra 1397	Atoxyl
B	45	98	49	30	(2)	(1)	5.6	5.5	<3	<3
C	1	5	4	<1	5	4	8.5	8.8	5.5	5.7

acetyl-β-methylcholine was relatively high, but lower than that for cock plasma. On the other hand, the carbon analogue of acetylcholine (3,3-dimethylbutyl acetate) was hydrolysed at a comparatively high rate (b_{30} 20). The hydrolysis rates with the *iso*amyl esters dropped off very rapidly with time although the initial rates were as high as with the triglycerides.

In contrast to cock plasma, the duck plasma gave two esterase peaks on electrophoresis. The main peak was due to a B-estrase which was sensitive to *iso*-OMPA (Fig. 2) and mipafox (pI_{50} 8.5). This esterase differed from the B-type esterases of the mammalian plasmata in being sensitive to 10^{-5} M physostigmine as is also characteristic of certain B-esterases of lower vertebrates (turtle, frog, pike). This propionyl-B-esterase was similar to a cholinesterase in regard to the susceptibility to inhibitors. However, a propionylcholinesterase (C) was found in a second slower-moving peak. Some of the esterase activity was also found in the B-esterase peak as determined by comparing the hydrolysis of propionylcholine and phenyl propionate by these fractions. Except for differences in sensitivity to physostigmine, the B- and C-esterases were best differentiated by using Astra 1397 (or atoxyl) which selectively inhibited the C-esterase in a concentration of 10^{-5} M.

Indoxyl acetate was hydrolysed by the B-esterase only.

Turtle, Testudo graeca (Fig. 3). The propionyl esters of all series of substrates were hydrolysed at the highest rate. Particularly high rates were observed with tripropionin and phenyl propionate. This plasma hydrolysed heroin

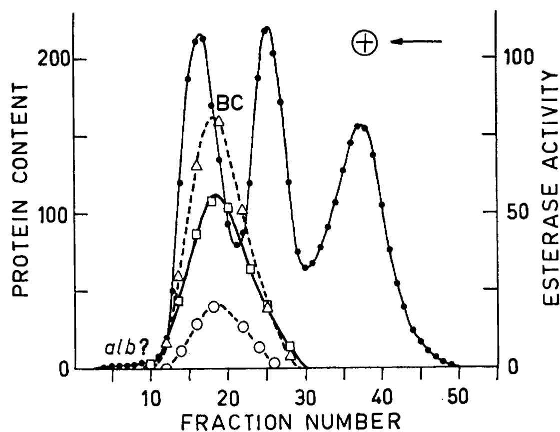

Fig. 3. Turtle plasma. Conditions as for Fig. 1. △— — —△, TPr (0.025); □———□, heroin (0.10); ○— — —○, PrCh (0.20).

Esterase component	PhPr	PhBu	TPr	PrCh	Heroin	pI_{50}	
						Phys	*iso*-OMPA
BC	210	60	320	10	55	6.3	6.6

Table 3. Susceptibility of turtle-plasma esterase to various inhibitors. *Cf.* Table 2 for details.

Inhibitor	Tri-propionin	Phenyl-propionate	Heroin	Propionyl-choline
Physostigmine	6.4	6.3	6.1	6.3
iso-OMPA	5.2	6.6	5.2	6.6
3318CT	<3.5	≈ 4.0	<3.5	≈ 4.0

(b_{30} 220) and the carbon analogue of acetylcholine (b_{30} 160) more rapidly than the choline esters.

Turtle plasma was almost free of albumin-like proteins, confirming a recent observation made by Cohen and Stickler [11]. Only one esterase peak was obtained on electrophoresis and this had about the same electrophoretic mobility as was found for most B-esterases of vertebrate plasmata. Most results obtained were consistent with the view that only one esterase was present in this plasma. Summation experiments with tripropionin, heroin and propionylcholine showed that all three substrates were probably split by the same esterase. This was also indicated by the inhibitory experiments, although the 50 % inhibition values (Table 3) obtained with the original plasma showed some discrepancies for the compounds *iso*OMPA and 3318CT.

The properties of this esterase were unique in many respects. It hydrolysed the propionates in the following order of decreasing rates: TPr > PhPr > AmPr > Heroin > PrCh. This esterase was sensitive to both physostigmine and the organophosphorus compounds used (Table 3). Therefore, it had the characteristics of both a B-and C-esterase (*cf.* Ref.[1]), and is the first example of a physostigmine-sensitive esterase hydrolysing non-choline esters more rapidly than choline esters. This esterase, therefore, may represent an intermediate stage in the phylogenetic evolution of plasma esterases (see further the third paper of this series [12]).

Frog, Rana temporaria (Fig. 4). The hydrolysis rate with phenyl esters varied considerably from animal to animal. The plasma from female frogs generally had much greater activity than that from male frogs against these esters. Phenyl acetate, for instance, was hydrolysed at about a 40 times higher rate by female plasma compared with male plasma. This sex difference was due to the presence of a propionyl- (or acetyl-) arylesterase (A-esterase; see Fig. 4, inserted graph) which was absent in the male frog plasmata investigated in this study. Heroin was hydrolysed at a higher rate by frog plasma (both sexes, b_{30} 25) than by most mammalian plasmata.

On electrophoresis the B- and C-esterases migrated close together, the latter having a somewhat lower mobility than the former, in contrast to most other plasma esterases of these types. A fast-migrating esterase was probably identical with the B-esterase of the main esterase peak as was demonstrated by substrate specificity and inhibitor experiments. This esterase was a propionylesterase which was more sensitive to physostigmine (pI_{50} 4.5) than the B-esterases of mammalian plasmata.

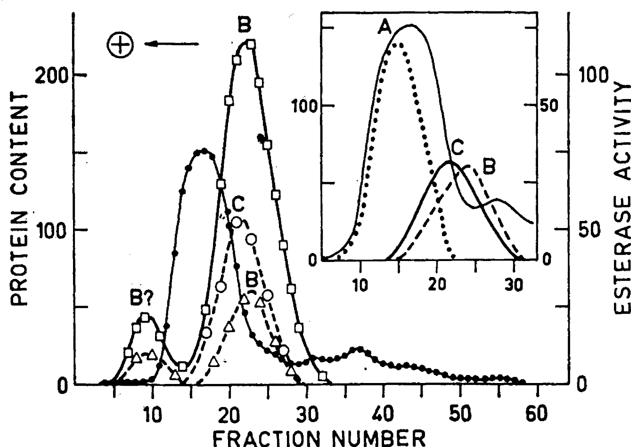

Fig. 4. Frog (male) plasma. Conditions as for Fig. 1. □——□, PhBu (0.40); △– – –△, TPr (0.40); ○– – –○, PrCh (0.40).

Esterase component	PhAc	PhBu	TPr	PrCh	BuCh	pI_{50}	
						Phys	*iso*-OMPA
B	210	30	7	0	0	4.5	6.2
B?	3	5	2	0	0	4.5	6.2
C	?	?	?	14	13	7.2	7.2

The insert (of the same scale as the main graph) is the electrophoretic pattern of the plasma from a female frog with high activity against phenyl esters (PhAc, $b_{30} > 5\,000$). ——, protein; ...., PhAc (0.02); – – – –, TPr (0.20); ——, BuCh (0.20).

The C-esterase of frog plasma was a propionylcholinesterase which hydrolysed butyrylcholine at a relatively high rate and was less sensitive to mipafox (pI_{50} 5.2) but more sensitive to atoxyl (pI_{50} 5.1) and quinine (pI_{50} 4.2) than are most other plasma cholinesterases. Atoxyl was a useful selective inhibitor of cholinesterase of this plasma, because both B- and A-esterases (when present) were resistant to this agent. Plasma cholinesterase of male frogs was responsible for about 30 % of the total hydrolysis of the phenyl esters.

The propionylcholinesterase was inhibited by high concentrations of propionylcholine and butyrylcholine with a substrate optimum of 7×10^{-3} M. In this respect this esterase resembles the group of acetylcolinesterases present in the erythrocytes of various animals.

South African clawed frog, Xenopus laevis (Fig. 5). The esterases present in the plasma sample analysed, collected from five females, differed greatly from those of the plasma of *Rana*. An arylesterase was absent because the

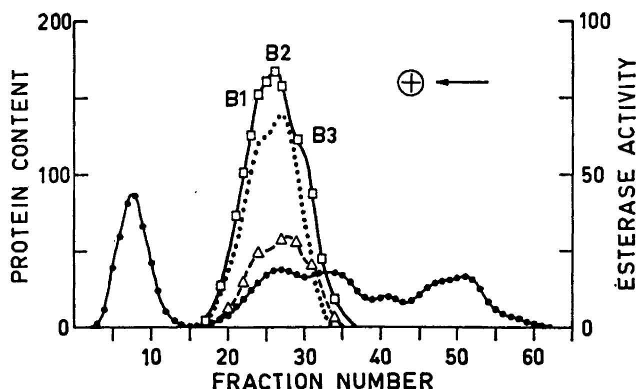

Fig. 5. Clawed-frog plasma. Conditions as for Fig. 1. □———□, PhBu (0.05); ...., PhAc (0.05); △———△, TPr (0.05).

Esterase component	PhAc	PhBu	AmBu	TPr	Tween	BuCh	pI_{50}	
							Phys	Mipafox
B1	120	150	3	50	2	0	3.5	6
B2	140	170	6	60	<7	0	3.5	6
B3	?	120	5	40	13	0	4.5	6
C	?	?	?	?	?	<1	7	7

hydrolysis of phenyl esters was completely inhibited by low concentrations of both mipafox and *iso*-OMPA. There were probably more than one B-type esterase with different substrate specificities. The complexity of the esterase peak obtained on electrophoresis was in conformity with this hypothesis. The peaks B1 and B2 could possibly be due to the same enzyme, but the esterase of peak B3 was characterized by relatively higher rate of Tween-20 hydrolysis and greater sensitivity to physostigmine than the B1- and B2-esterase fractions. The B-esterases were all of the butyryl- or propionyl-esterase type and hydrolysed heroin at high rates (b_{30} per 0.1 ml plasma, 110).

The C-esterase, present in too low concentration to be detected after electrophoresis, was a butyrylcholinesterase which was highly sensitive to Astra 1397 ($pI_{50} \approx 5$) as are most of the plasma butyrylcholinesterases.

This plasma contained in high concentration a protein which migrated faster on electrophoresis than most albumin-like proteins of other plasmata.

Pike, Esox lucius (Fig. 6). Plasma from this fish hydrolysed triglycerides more rapidly than phenyl esters, tripropionin being split at the highest rate. The main esterase present was a B-esterase being highly sensitive to mipafox ($pI_{50} \approx 9$) and less so to physostigmine (pI_{50} 4.8) when tested with tripropionin,

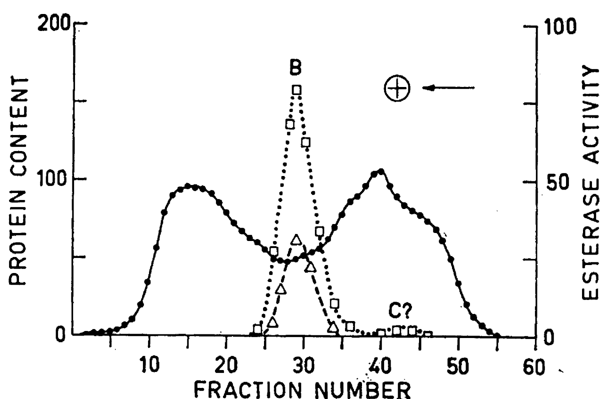

Fig. 6. Pike plasma. Conditions as for Fig. 1. □······□, PhAc (0.10); △– – –△, AmBu (0.10).

Esterase component	PhAc	PhBu	AmBu	TPr	AcCh	pI_{50}	
						Phys	Mipafox
B	80	75	30	100	0	4.8	≈ 9
C	?	0	0	0	< 1	6.5	≈ 4

phenyl acetate and phenyl butyrate respectively as substrates. This enzyme hydrolysed heroin at a low rate (b_{30} 7) in contrast to the B-esterases of cod and eel plasmata, and the carbon analogue of acetylcholine at a relatively high rate (b_{30} 34).

The C-esterase present in low concentration was an acetylcholinesterase which was much less sensitive to mipafox than the B-esterase. In this respect the pike cholinesterase was similar to an acetylcholinesterase of higher animals.

Cod, Gadus callarias (Fig. 7). An acetyl-B-esterase was characteristic of this plasma. Heroin was hydrolysed at a high rate (b_{30} 220), and mipafox was an inhibitor but not *iso*-OMPA. The esterase appeared as a uniform peak on electrophoresis.

An acetylcholinesterase was present but in too low concentration to be detected in the fractions after electrophoresis.

Eel, Anguilla anguilla (Fig. 8). This plasma was characterized by its unusually high activity in hydrolysing aromatic esters and triglycerides, especially those of butyric acid. This material seems to be the richest animal source of "lipase" activity ever described. It is 100 times more active than human plasma in hydrolysing these esters. Heroin was hydrolysed at a high rate (b_{30} 210) and so also was Tween 20 (b_{30} 60). The high "lipase" activity tested with tributyrin has been reported previously [13].

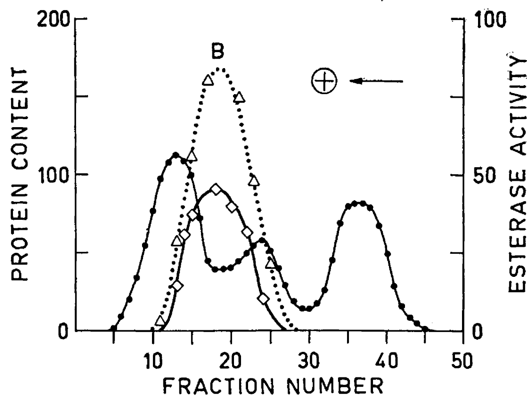

Fig. 7. Cod plasma. Conditions as for Fig. 1. △·······△, TAc (0.20); □———□, heroin (0.20).

Esterase component	PhAc	*p*-Nitro-PhAc	TAc	Heroin	AcCh	pI_{50}	
						Phys	Mipafox
B	60	40	42	23	0	≈3.5	5.3
C	?	?	?	?	<1	≈6	—

The main esterase present was a butyryl- or propionylesterase (B1), which hydrolysed the aromatic esters at a higher rate than the triglycerides. This enzyme was highly sensitive to mipafox and *iso*-OMPA (see legends to Fig. 8), and as many esterases of this type present in the plasmata of lower vertebrates it was relatively sensitive to physostigmine. A minor esterase peak with a lower electrophoretic mobility was also observed. It was due to an esterase which differed from the main esterase in hydrolysing the acetates more rapidly than the butyrates, in being much less sensitive to mipafox and *iso*-OMPA and highly resistant to physostigmine. This enzyme has to be regarded as a B-esterase, although the aliphatic esters were hydrolysed at a lower rate than the aromatic ones.

As with most fish plasmata, eel plasma contained a C-esterase, the specificity and sensitivity to inhibitors of which were characteristic of an acetylcholinesterase. High concentration of acetylcholine inhibited the activity.

Wrasse, Labrus berggylta (Fig. 9). The plasma from females contained a B-esterase which hydrolysed butyrates and propionates at a higher rate than acetates. It was similar to the B2-esterase of eel plasma in being sensitive to mipafox but not to *iso*-OMPA. Tween 20 was hydrolysed at a relatively high rate by this enzyme (b_{30} 100). Choline esters were hydrolysed at very low rates, due probably to an enzyme similar to an acetylcholinesterase.

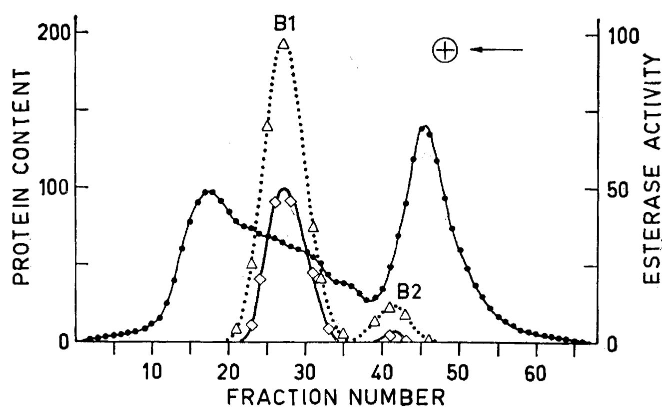

Fig. 8. Eel plasma. Conditions as for Fig. 1. △·······△, TAc (0.05); ◇———◇, heroin (0.1).

Esterase component	PhAc	PhBu	AmBu	TAc	TPr	TBu	Heroin	AcCh
B1	2 000	5 000	65	190	1 580	1 500	50	0
B2	30	1	0	21	7	2	3	0
C	?	?	?	<1	?	?	?	<1

pI_{50}				
Phys	Mipafox	*iso*-OMPA	Astra 1397	3318CT
3.8	7.5	7.1	4.5	3.5
<3	3.2	<3	<3	<3
7.7	—	4.1	—	5.2

The plasmata of Labrides are characterized by the presence of a blue-coloured dye [14,15]. On electrophoresis, this dye of protein nature was easily separated from the other three main protein constituents by its comparatively low mobility. The coloured protein was probably a chromoprotein which gave absorption maxima at 390, 600 and 645 mμ. Part of the dye with the same absorption maxima was found also in two other protein fractions (see Fig. 9).

The four protein peaks obtained on electrophoresis were recently observed also by Fine and Drilhon [16] on electrophoresis on paper and gelatine.

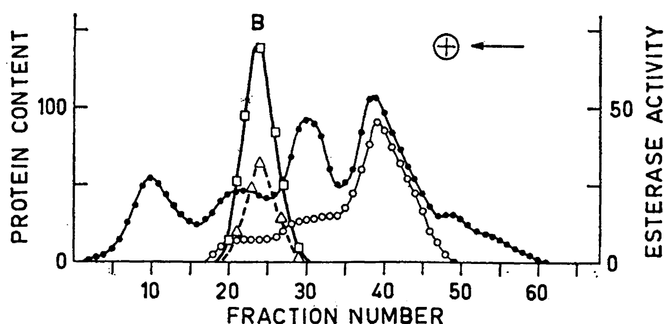

Fig. 9. Wrasse plasma. Conditions as for Fig. 1. □———□, PhBu (0.05); △– – – –△, TPr (0.1); ○———○, blue colour measured spectrophotometrically at 645 mμ.

Esterase component	PhAc	TPr	Tween	AcCh	pI_{50}			
					Phys	Mipafox	*iso*-OMPA	3318CT
B	140	32	30	0	<3	4.1	<3	3.4
C	0	0	0	<1	≈ 6	≈ 6	—	—

Frogfish, Lophius piscatorius (Fig. 10). A B-esterase was present in high concentration and had the properties of an acetylesterase. This esterase hydrolysed heroin at a higher rate (b_{30} 535) than any other plasmata studied in this series. In contrast to most other esterases of this type, *para*-substitution in phenyl acetate with a nitro group increased the enzymic hydrolysis. Mipafox was an inhibitor but not *iso*-OMPA. Like the B-esterase of *Labrus* plasma, the frog-fish esterase was sensitive to physostigmine and 3318CT in 10^{-4} M or higher concentrations. An acetylcholinesterase was probably present in low concentration.

This plasma was characterized by the very low content of albumin-like proteins.

Other teleostians. Blood plasma samples from sea-scorpion (*Cottus scorpius*) and mackerel (*Scomber scomber*) were also analysed. Both plasmata contained a B-esterase in low concentration which was of a butyryl- or propionylesterase type. Choline esters were hydrolysed at very low rates, due to the presence of an acetylcholinesterase. The low content of such an esterase in teleostian plasmata was reported previously [5,17].

The electrophoretic patterns showed three or four protein peaks, the mackerel plasma having higher concentration of fast-running proteins than most other fish plasmata studied. The green dye characteristic of the Cottied plasmata was probably a chromoprotein which migrated electrophoretically more rapidly than the other main plasma proteins. The electrophoretic patterns of these two plasmata will be published in a following paper [12].

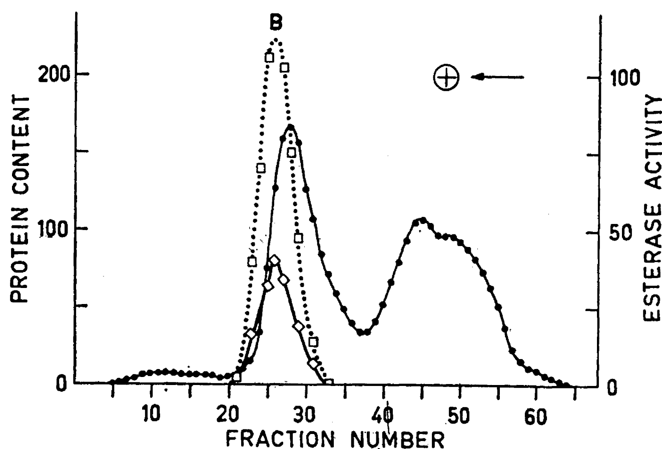

Fig. 10. Frogfish plasma. Conditions as for Fig. 1. □······□, PhAc (0.05); ◇——◇, heroin (0.025).

Esterase component	PhAc	TAc	Heroin	pI_{50}			
				Phys	Mipafox	*iso*-OMPA	3318CT
B	225	155	160	4.0	5.5	<3	3.4
C	?	?	?	≈ 7	—	—	—

Spiny dogfish shark, Squalus acanthias. The plasma sample obtained from this Elasmobranch had very low esterase activity, too low to be detected after electrophoresis. The activity was due to a propionyl-B-esterase and an acetylcholinesterase. The low content of the latter enzyme was previously reported [5,17]. The protein curve obtained on electrophoresis is shown in Fig. 11.

Hagfish, Myxine glutinosa. The sample collected from several animals had negligible esterase activity when tested with most substrates. Aromatic esters were hydrolysed at very low rates and this reaction was probably due to an acetyl- or propionyl-B-esterase. A cholinesterase was probably not present. Fig. 11 shows the electrophoretic pattern of the protein components which were present in low concentrations (3.5 mg of total protein per 100 μl plasma).

The low lipase activity of the plasma from another Cyclostome (*Petromyzon marinus*) was noticed previously [13].

A discussion of the results reported above in relation to those of the first report of this series will be presented in a concluding paper [12].

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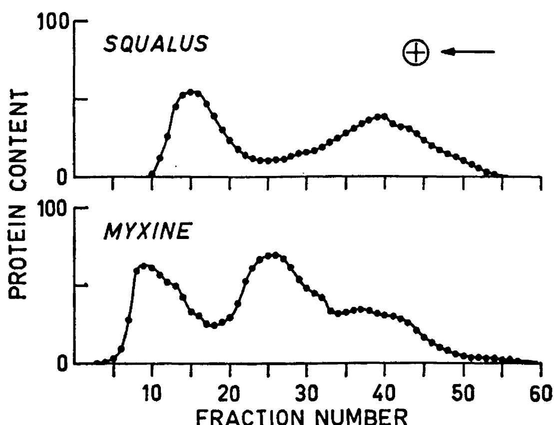

Fig. 11. Plasmata from spiny dog-fish shark and hagfish. Conditions as for Fig. 1. Esterase activities were too low to be detected after electrophoresis.

Species	Esterase component	pI_{50}
		Phys
Squalus	B	4 (PhAc)
	C	< 7.0 (AcCh)
Myxine	B?	< 4 (*p*-nitro-PhAc)

marine fish, and I wish to express my sincere gratitude to Dr. Gunnar Gustafson for placing the facilities of the station at my disposal. I also thank Dr. Claes-Henry Hansson, Dr. Bertil Olsson, Dr. Torsten Fredriksson, Assistent Johan Lundin and Dr. Tore Hultin for blood samples of various animals. Grateful acknowledgements for the kind supply of chemicals are due to Dr. Lars-Erik Tammelin (for *iso*-OMPA, mipafox, and 3,3-dimethylbutyl acetate), Dr. Richard Dahlbom (for the compound Astra 1397), Dr. F. C. Copp (for the compound 62C47), and Dr. J. Jacob (for the compound 3318CT).

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